LESSONS LEARNED

When is Protein Binding Important?*

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ABSTRACT: The present paper is an ode to a classic citation by Benet and Hoener (2002. Clin Pharm Ther 71(3):115–121). The now classic paper had a huge impact on drug development and the way the issue of protein binding is perceived and interpreted. Although the authors very clearly pointed out the limitations and underlying assumptions for their delineations, these are too often overlooked and the classic paper's message is misinterpreted by broadening to cases that were not intended. Some members of the scientific community concluded from the paper that protein binding is not important. This was clearly not intended by the authors, as they finished their paper with a paragraph entitled: "When is protein binding important?" Misinterpretation of the underlying assumptions in the classic work can result in major pitfalls in drug development. Therefore, we revisit the topic of protein binding with the intention of clarifying when clinically relevant changes should be considered during drug development. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3458–3467, 2013

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INTRODUCTION

Over 10 years ago, Benet and Hoener[1] published a citation classic paper entitled, "Changes in plasma protein binding have little clinical relevance". In this paper, the authors elegantly presented the case that changes in plasma protein binding will usually not affect the exposure of free, pharmacologically active drug. They convincingly concluded that drug–drug interactions (DDIs) due to displacement from plasma protein binding sites may still occur but will not result in changes in unbound drug exposure and have consequently little clinical relevance. Before the Benet and Hoener publication, Clinical Pharmacology textbooks included a chapter on DDIs caused by protein binding displacement and presented the warfarin–phenylbutazone interaction as a typical example.[2] In this example, a causal link between *in vitro* experiments and clinical bleeding events was established, arguing that coadministration of phenylbutazone leads to an increase in free, active warfarin concentrations due to protein binding displacement. However, Benet and Hoener provided in their paper a theoretical argument, which showed that this conclusion was wrong and that protein binding displacement of warfarin does not result in free, unbound drug concentrations and pharmacological activity. In fact, it turned out that there is a second DDI involving enzyme inhibition by phenylbutazone that was responsible for the resulting increase in unbound drug exposure.[3–5]

Benet and Hoener were also very detailed in pointing out the assumptions and limitations of their conclusions. Unfortunately, the title of the paper was—and still is—sometimes misinterpreted by those who overlook the detailed presentation of the underlying assumptions and limitations of the conclusions that are discussed in the seminal work.[3,4] The misinterpretation is to erroneously think that protein binding in general is of little relevance. Benet and Hoener anticipated this wrong interpretation and stated: "This conclusion should not be extrapolated to suggest that measurements of protein binding are not important in drug development." These limitations

Abbreviations used: AL, albumin; AAG, alpha-1-acid glycoprotein; CL_H, hepatic clearance; $CL_{int,in\ vitro}$, intrinsic clearance *in vitro*; $CL_{int,in\ vivo}$, intrinsic clearance *in vivo*; DDI, drug–drug interaction; fu_b, unbound fraction in blood; fu_{inc}, unbound fraction in incubation; fu_{liver}, unbound fraction in liver; fu_p, unbound fraction in plasma; $fu_{p\text{-}app}$, unbound fraction in plasma apparent; IVIVE, *in vitro–in vivo* extrapolation; K_i, inhibition constant; $K_{i,u}$, unbound inhibition constant; MIC, minimum inhibitory concentration; PLR, plasma-to-liver concentration ratio; Q, liver blood flow; R_{BP}, blood-to-plasma ratio.

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Dedicated to Leslie Z. Benet

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are discussed in the last paragraph of their paper entitled "When is protein binding important?". It is the purpose of this paper to revisit these limitations and expand on some protein-binding related aspects that can be major pitfalls in drug development if ignored.

HIGH-EXTRACTION DRUGS AFTER PARENTERAL ADMINISTRATION

Benet and Hoener pointed out that there is one exception to the rule that unbound drug exposures are not significantly altered by changes in plasma protein binding, that is, high-extraction drugs following parenteral administration.[1] For high extraction drugs with hepatic clearance, total drug clearance is approximately equal to liver blood flow and, hence, independent of plasma protein binding. Average total drug concentrations and total area under the curve (AUC) will not change if protein binding is altered. However, the scenario changes when considering unbound drug concentrations as they are proportional to the fraction unbound in plasma and, hence, sensitive to changes in plasma protein binding. One example of this concept is the inhaled corticosteroid, ciclesonide. The drug is an inactive prodrug that is administered by inhalation and quickly converted into its active metabolite desciclesonide.[6–8] Ciclesonide's clearance is close to liver blood flow, in spite of its very high plasma protein binding of approximately 99%. Consequently, it is a clear example illustrating that high plasma protein binding does not automatically result in clearance restrictions. This example further shows that intrahepatic reequilibration between bound and free drug occurs so fast that the vast majority of drug can be metabolized as the blood crosses the liver. Although protein binding is not rate-limiting for hepatic clearance for this type of drug, it still controls the magnitude of the unbound plasma concentrations. Concentrations are much lower than those of other inhaled corticosteroids with comparable clearance,[6–8] which leads to much lower systemic side effects as indicated by lower suppression of endogenous cortisol as well as lack of growth retardation in children.[9]

WHAT ABOUT VOLUME OF DISTRIBUTION?

Although it is true that the total average steady-state concentration after multiple dosing as well as the total AUC will not change when clearance is unaltered, the shape of the plasma profile will be different if the volume of distribution changes due to protein binding changes. This change in volume of distribution will depend on the relationship of drug binding in plasma and in tissues. If the fraction bound in plasma increases more than that in tissues, the volume of distribution will go down. As a result, total peak plasma concentrations will increase, the half-life will be shorter and total trough concentration in plasma will be lower. The opposite will be true if the fraction bound in the tissues increases more than that in plasma: the volume of distribution will increase, total peak plasma concentrations will be lower, the half-life will be longer and total trough concentrations in plasma will be elevated. In all of these scenarios the total average steady-state concentration and the total AUC will remain unaltered if clearance does not change. A more detailed analysis of these scenarios can be found elsewhere.[10] Depending on the relationship between pharmacokinetics and pharmacodynamics (PK/PD), these different plasma drug profiles may result in different clinical outcomes as it is not always the AUC that correlates best with clinical outcome. For example, it is well known that the antiinfective activity of beta-lactam antibiotics correlates best with the time that the unbound plasma concentrations remain over the respective minimum inhibitory concentration (MIC) of the microorganism that is to be eradicated rather than the AUC.[11]

MISINTERPRETATION OF DRUG LEVEL MONITORING

Therapeutic drug monitoring (TDM) is another potential source for misinterpreting the impact of plasma protein binding as was pointed out already in great detail by Benet and Hoener.[1] TDM is usually performed and therapeutic target ranges are defined on the basis of total (free + bound) plasma concentrations. Although it is now well established that only the unbound drug concentration is responsible for the PD activity.[10–13] In most cases, reporting total drug levels does not pose a problem as total concentrations (e.g., 10–20 mg/L for total phenytoin) directly correspond to unbound target concentrations (e.g., 1–2 mg/L for unbound phenytoin based on a fraction bound of 0.9) if the fraction bound in plasma is constant over the therapeutic range. However, if protein binding changes due to disease or DDIs, this relationship is no longer valid. Although the unbound target range is still valid, the total target range will differ. The simple solution in this situation is to monitor unbound drug concentrations, which will give unambiguous values to directly compare with the desired unbound target range. However, not all clinical laboratories are prepared or willing to measure unbound concentrations. As a second-best solution for this situation, Winter proposed a work-around using the measured albumin concentration to estimate the degree of plasma protein binding and adjust the measured total phenytoin concentration in a patient with lowered albumin concentrations to the respective concentration in a patient with normal albumin concentrations so that the number then can be compared with the established target range for total phenytoin concentrations.[14]